

The calceolus, a sensory structure of gammaridean amphipods (Amphipoda: Gammaridea)

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Introduction

The calceolus is a microscopic external surface structure, presumed to serve a sensory function, found on the antennae of a select group of amphipods belonging to the suborder Gammaridea. It occurs in only about 10 per cent of the known gammaridean species, and is absent elsewhere in the Crustacea. First noted by Milne-Edwards in 1830 and referred to as the 'cupule membraneuse', it later acquired the name 'calceolus' because of its slipper-shaped profile when viewed under a microscope. A good account of contemporary knowledge was provided by Blanc (1883, 1884).

Despite this early recognition, calceoli have since received only limited attention from taxonomists and physiologists, and remain largely enigmatic inconsistently-documented structures. Their occurrence is uncertain in many species, they are poorly understood in terms of morphology and ontogenetic development, and their precise function has yet to be established. The small size of calceoli (20–300 μm) is the probable explanation for this lack of attention, since good resolution of their intricate surface structure is almost impossible using conventional light microscopy, and they are easily overlooked at the lower magnifications often used in figuring antennae for taxonomic work. A few attempts have been made by taxonomists to draw calceoli at high magnification under a light microscope, and some idea of the general profile and surface pattern has been obtained, but the true three-dimensional complexity of the structure cannot be appreciated. Some of the earliest drawings of calceoli are as good as or better than most illustrations in recent literature.

Calceoli have not always been reliably distinguished from aesthetascs. These also occur frequently on amphipod antennae but have a much simpler structure. Confusion has been especially noticeable in taxonomic work on freshwater amphipods which may have aesthetascs of unusually large size. Unlike calceoli, aesthetascs are found widely throughout the Crustacea, and are thought to function as chemoreceptors. Aesthetascs in amphipods generally have a very simple spatulate shape and are restricted to the flagellum of antenna 1. The structurally more bizarre calceoli are found on antenna 2 or both antenna 1 and 2, but not on antenna 1 alone. In some species, as for example *Eusirus antarcticus* Thomson, calceoli and aesthetascs occur together on the flagellar articles of the same individual dispelling any thoughts that calceoli and aesthetascs might simply be variants of the same surface structure. In *E. antarcticus* the calceolus is about one-third the length of the aesthetasc.

We have assembled a considerable amount of data on the occurrence and distribution of calceoli amongst amphipods but have found surprisingly little ecological or biological pattern in this information. For certain, calceoli do not occur outside the suborder Gammaridea, but of the 80 or so families of gammarideans presently recognised only 19

Table 1 Superfamilies and families of gammaridean amphipods (after Bousfield, 1978). Calceoliferous families are shown in bold capital letters.

Phoxocephaloidea	Oedicerotoidea	Pardaliscoidea
PHOXOCEPHALIDAE	OEDICEROTIDAE	Pardaliscidae
UROTHOIDAE		Stilipedidae
PLATYISCHNOPIDAE	Leucothoidea	Hyperiopidae
	Pleustidae	Astyridae
Lysianassoidea	Laphystiopsidae	Vitjazianidae
LYSIANASSIDAE	Amphilochidae	
	Leucothoidae	Liljeborgioidea
Pontoporeioidea	Anamixidae	Liljeborgiidae
HAUSTORIIDAE	Maxillipiidae	Sebidae
PONTOPOREIIDAE	Colomastigidae	Salentinellidae
	Pagetinidae	
Gammaroidea	Nihotungidae	Dexaminoidea
GAMMARIDAE	Stenothoidae	Atylidae
ACANTHOGAMMARIDAE	Cressidae	Anatylidae
ANISOGAMMARIDAE	Thaumatelsonidae	Lepechinellidae
MESOGAMMARIDAE		Dexaminidae
Gammaroporeiidae		Prophliantidae
Macrohectopidae	Talitroidea	
Typhlogammaridae	Hyalidae	Ampeliscoidea
Pontogammaridae	Hyalellidae	Ampeliscidae
	Talitridae	
Crangonyctoidea	Ceinidae	Melphidippoidea
CRANGONYCTIDAE	Dogielinotidae	Melphidippidae
NEONIPHARGIDAE	Najnidae	
PARAMELITIDAE	Eophliantidae	Melitoidea
	Phliantidae	Hadziidae
Niphargoidea	Temnophliantidae	Melitidae
Niphargidae	Kuriidae	Carangoliopsidae
Bogidielloidea	Stegocephaloidea	Corophioidea
Bogidiellidae	Stegocephalidae	Photidae
	Acanthonotozomatidae	Isaeidae
Eusiroidea	Ochlesidae	Ischyroceridae
EUSIRIDAE	Lafystiidae	Ampithoidae
PONTOGENEIIDAE		Biancolinidae
CALLIOPIIDAE		Aoridae
GAMMARELLIDAE		Cheluridae
AMATHILLOPSIDAE		Corophiidae
Bateidae		Podoceridae
Paramphithoidae		

contain calceoliferous species (Table 1), and these are restricted to just 7 of the 19 superfamilies (as proposed by Bousfield (1978) in a recent revision of the group). Even within these families the calceoli are far from uniformly distributed; some genera are entirely non-calceoliferous, others have both calceoliferous and non-calceoliferous species. Ecologically, the calceoliferous species show no special pattern—they may occur in marine, brackish water or freshwater (including hypogean) habitats, from shallow to abyssal depths, in polar, temperate or tropical regions, and may be active swimmers, or burrowers, or live in algae. We could find no obvious correlation of the presence or absence of calceoli with behavioural patterns. An additional dimension of variability is suggested by recent ecological work which affirms that calceoli may be present or absent in different populations of the same species, or from different samples of the same population taken at different seasons of the year (Minkley & Cole, 1963; Cole, 1970; Goedmakers, 1972; Croker & Gable, 1977), although we have

some reservations as to the universality of these statements. Jazdzewski (1977) suggests that other considerations such as the appearance of calceoli only in males of a certain age may also account for apparent variability of occurrence within species' populations.

A checklist of all known calceoliferous amphipod species is given in a recent paper by Hurley (1980). This tabulation includes only those species that have actually been described or figured in the literature as having calceoli, excluding any that are calceoliferous by inference alone (e.g. generic diagnosis). The list comprises 584 species and subspecies belonging to 134 different genera, from an estimated 5000 species and an estimated 1000 genera in the Gammaridea as a whole. Within the 19 calceoliferous families, of approximately 375 genera and 2000 species, the proportion of species possessing calceoli is a little less than one-third.

Hurley's compilation shows up some trends in the location of the calceoli on the antennae in the different families. In haustoriids, phoxocephalids and lysianassids, only the male has calceoli which may occur on antenna 1 and antenna 2, although in lysianassids they are absent from peduncular articles. Gammarids, acanthogammarids, anisogammarids and mesogammarids have a few species with calceoliferous females, but calceoli are typically restricted to the flagellum of antenna 2 in males. The crangonyctids have a similar pattern to the 4 gammaroid families above except that the calceoli occur on the peduncle as well as the flagellum of antenna 2. Eusiroids (Eusiridae, Pontogeneiidae, Calliopidae, Gammarellidae, Amathillopsidae) are commonly calceoliferous in both sexes and on both antennae.

The function of calceoli has received very little direct attention and is far from resolved. They have variously been considered organs for clasping, copulation, and taste, and more recently linked with pheromone reception (Dahl *et al.*, 1970) but only the latter hypothesis is supported by direct experimental evidence. However, from structural and other evidence we would argue against a chemosensory role for calceoli. We believe the structural complexity of the calceoli involves some form of sound, vibration or pressure wave sensitivity.

Scanning electron microscopy was used to examine the calceoli of more than 60 different amphipod species in some 40 genera representing most of the calceoliferous families. We looked first at the morphology of a wide range of calceoli and applied this information to the problem of function. An unexpected bonus, following from the recognition of distinct structural designs amongst the calceoli, has been the rewarding prospect of using them as indicators of phylogenetic affinity.

Material and methods

All the scanning work for this study was carried out in the E.M. Unit of the British Museum (Natural History) using either a Cambridge 2A or a Stereoscan 600. Satisfactory results were obtained with antennal preparations that were simply oven dried before coating, although this was later replaced by routine critical point desiccation followed by sputter coating with gold. A variety of different methods for fixing preparations to stubs were tried and most proved adequate, but use of a thin film of Araldite was eventually adopted as the simplest and most effective. All source material from which dissections were made came either from the collections of the BM(NH) or from the N.Z. Oceanographic Institute. Some of this material had been in preservative for many years and had rather too much attached debris for high resolution photomicrography, but in all instances the basic configuration of the calceolus was quite clear, and material preserved in spirit for over a century still gave useful, if not spectacular, results.

Results

The calceoli of the sixty or so species examined showed considerable morphological diversity from the relatively simplistic condition found in *Phoxocephalus* and *Urothoe*, to

the highly complex structures in *Eusirus*, *Amathillopsis*, *Chosroes*, and others. Despite this architectural variety, a certain basic design was evident throughout.

The typical calceolus (Figs 1a, 3a) has two surface components, which we have designated the *proximal* (p.e.) and *distal elements* (d.e.), more or less closely attached to the basal *receptacle* (r.), and a slender *stalk* (st.). The *distal element* is characterised by a series of ridges or annulations, or may comprise a number of separate or partially overlapping plates. The *proximal element*, in contrast, is a single component, either a concave crescent-shaped plate closely applied to the proximal margin of the distal element, or a discrete circular cup that sits freely on the receptacle attached only by a narrow base.

In the majority of calceoli examined, except those of phoxocephalids, urothoids and crangonyctids, there is a large bulbous swelling or *bulla* (b.) at the proximal end of the receptacle close to the attachment of the stalk. In a few of the eusirid calceoli studied the proximal element had been dislodged during the preparation revealing a circular opening in the receptacle through which the base of the proximal dish appeared to connect to the underlying bulla.

Within our sample we have been able to recognise just 9 distinct structural types, and have described and illustrated each of these nine different designs, and listed those species allocated to each group. The 9 categories are designated after the significant family component; gammarid, bathyporeid, lysianassid, pontogeneiid, eusirid, gammarellid, oedicerotid, phoxocephalid and crangonyctid.

1. Gammarid (Fig. 1a–c)

Gammarus duebeni Liljeborg

Gammarus locusta (L.)

Gammarus pulex (L.)

Eulimnogammarus fuscus (Dybowsky)

Eulimnogammarus verrucosus (Gerstfeldt)

Echinogammarus veneris (Heller)

Eogammarus confervicolous (Stimpson)

Odontogammarus calcaratus (Dybowsky)

Micruropus talitroides (Dybowsky)

Micruropus vortex (Dybowsky)

*Micruropus wahl*i (Dybowsky)

The gammarid calceolus represents one of the simplest configurations. The proximal element forms a weakly concave crescentic plate closely applied along its inner margin to the distal element. The distal element usually has well defined transverse banding ranging from an observed maximum of 25–30 bands in *Eulimnogammarus verrucosus* and *Eogammarus confervicolous*, through 10 in *Micruropus wahl*i to as few as 2–3 poorly defined bands in *Micruropus vortex* and *M. talitroides*. High magnification of the distal element reveals that the banded markings are not simple ridges but a series of closely overlapping transverse plates, typical of the distal elements of almost all calceoli investigated.

Gammarid calceoli are usually confined to males, are typically few in number, and there is only one calceolus on each flagellar article.

2. Bathyporeid (Fig. 1d)

Bathyporeia guilliamsoniana (Bate)

Bathyporeia pilosa Lindström

Bathyporeia sarsi Watkin

Zaramilla kergueleni Stebbing

The bathyporeid calceolus is basically similar to the gammarid-type but is characterised by short tentacle-like projections along the posterior margin of the proximal element. The proximal element is a quite small shallow crescent shaped plate in close contact with the banded distal element. The banding is very much as in the gammarid pattern, and varies

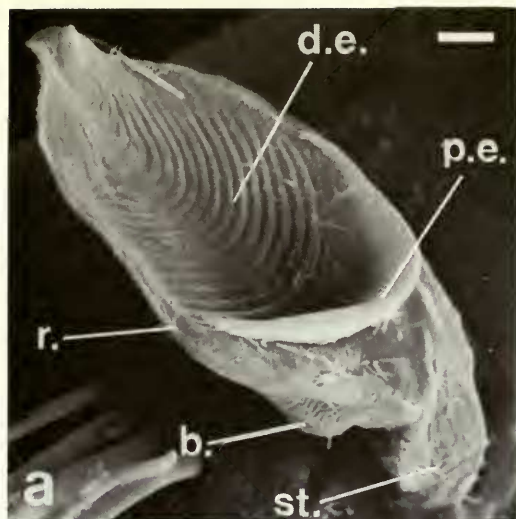


Fig. 1 a, *Gammarus pulex*, d.e., distal element; p.e., proximal element; r., receptacle; b., bulla; st., stalk; b., *Micruropus wahlí*; c, *Eulimnogammarus verrucosus*; d, *Bathyporeia sarsi*; e, *Oediceroides lahillei*; f, *Parawaldeckia thomsoni*. Bar scales = 10 μ m.

from 7 bands in *Bathyporeia sarsi* to about 25 in *Zaramilla kergueleni*. In both *Zaramilla* and *Bathyporeia* species the calceoli are present on the flagellum of antenna 1 and antenna 2 in the male only, and there is only one calceolus on any one flagellar article.

3. Lysianassid (Figs 1f, 2a–f)

Amaryllus macrophthalma Haswell
Cheirimedon similis Thurston
Hippomedon denticulatus (Bate)
Hippomedon holbolli (Kröyer)
Lepidepcreum cingulatum Barnard
Orchomene plebs (Hurley)
Parawaldeckia thomsoni (Stebbing)
Pseudorchomene coatsi (Chilton)
Socarnes vahli (Kröyer)
Tryphosella kergueleni (Miers)
Uristes gigas Dana
Waldeckia obesa (Chevreux)

The two surface elements of the lysianassid calceolus are more or less flattened and partially overlap, the distal element uppermost. The proximal element ranges in shape from a small crescent in *Hippomedon holbolli* to an almost circular disc in *Waldeckia obesa*, *Parawaldeckia thomsoni* and *Cheirimedon similis*. The distal element has weak surface banding which radiates sublongitudinally from a point close to the proximal margin in *Waldeckia*, *Orchomene*, *Parawaldeckia*, *Pseudorchomene*, *Lepidepcreum* and *Amaryllus*. In *Hippomedon*, *Tryphosa* and *Socarnes*, it appears quite smooth. The proximal element is only weakly concave with a slightly raised outer margin. The distal element is typically flattened, and is rather membraneous at the distal free margin. In lateral view, both surface elements rest rather freely on the receptacle with a small area of attachment near the centre. To support both surface elements the receptacle is elongated and extends almost to the distal margin of the distal element. The bulla is always well developed in the lysianassid calceolus.

One surprising and rather anomalous exception to the typical lysianassid design is found in *Uristes gigas* (Fig. 2f) which has the distal element of the calceolus strongly banded in concentric ridges that have their centre of origin close to the distal margin. This configuration has some resemblance to the pontogeneiid-type described below.

4. Pontogeneiid (Fig. 3a–d)

Apherusa jurinei (Milne-Edwards)
Bovallia gigantea Pfeffer
Calliopius laeviusculus (Kröyer)
Eusiroides monoculoides (Haswell)
Eusiropsis riisei Stebbing
Eusiroides stenopleura Barnard
Halirages fulvocinctus (Sars)
Halirages mixtus Stephensen
Paracalliopie fluviatilis (Thomson)
Paramoera gregaria (Pfeffer)
Pontogeneia sp.

The pontogeneiid calceolus is constructed along similar lines to the lysianassid-type, but is typically more robust with a distinctly concave proximal element and a large strongly banded distal element. The proximal element has the shape of an almost complete cup in *Bovallia gigantea* and the 5 species of *Eusiroides*, *Eusiropsis* and *Halirages*, and is larger than the distal element in *E. monoculoides*, subequal in *E. stenopleura*, and smaller in *E. riisei*. In contrast, a relatively small crescent-shaped proximal element is found in *Calliopius laeviusculus*, *Apherusa jurinei* and *Paramoera gregaria*, partially overlapped by the larger

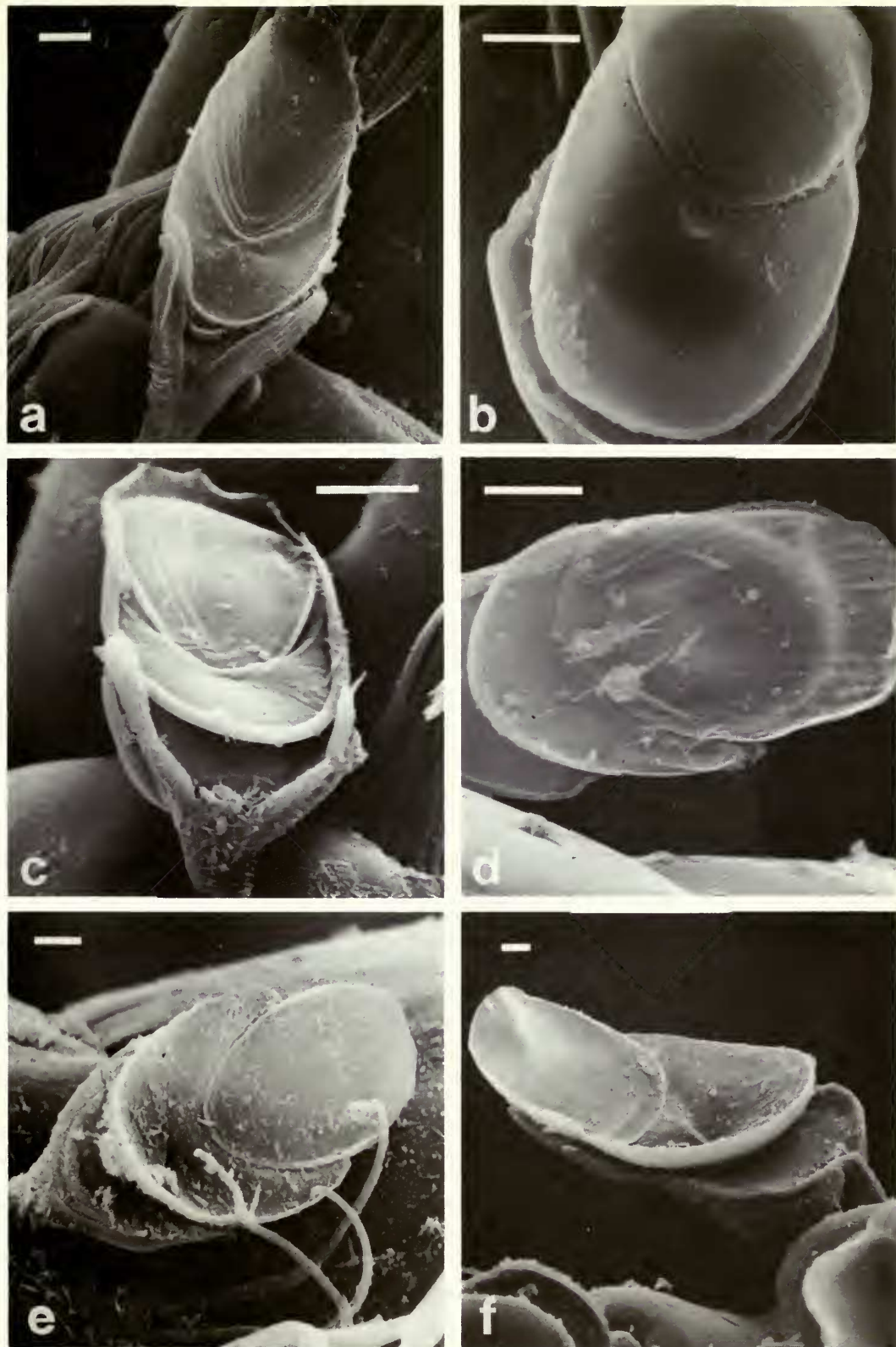


Fig. 2 a, *Orchomene plebs*; b, *Waldeckia obesa*; c, *Pseudorchomene coatsi*; d, *Hippomedon holbolli*; e, *Socarnes vahli*; f, *Uristes gigas*. Bar scales a-e = 10 μ m, f = 20 μ m.

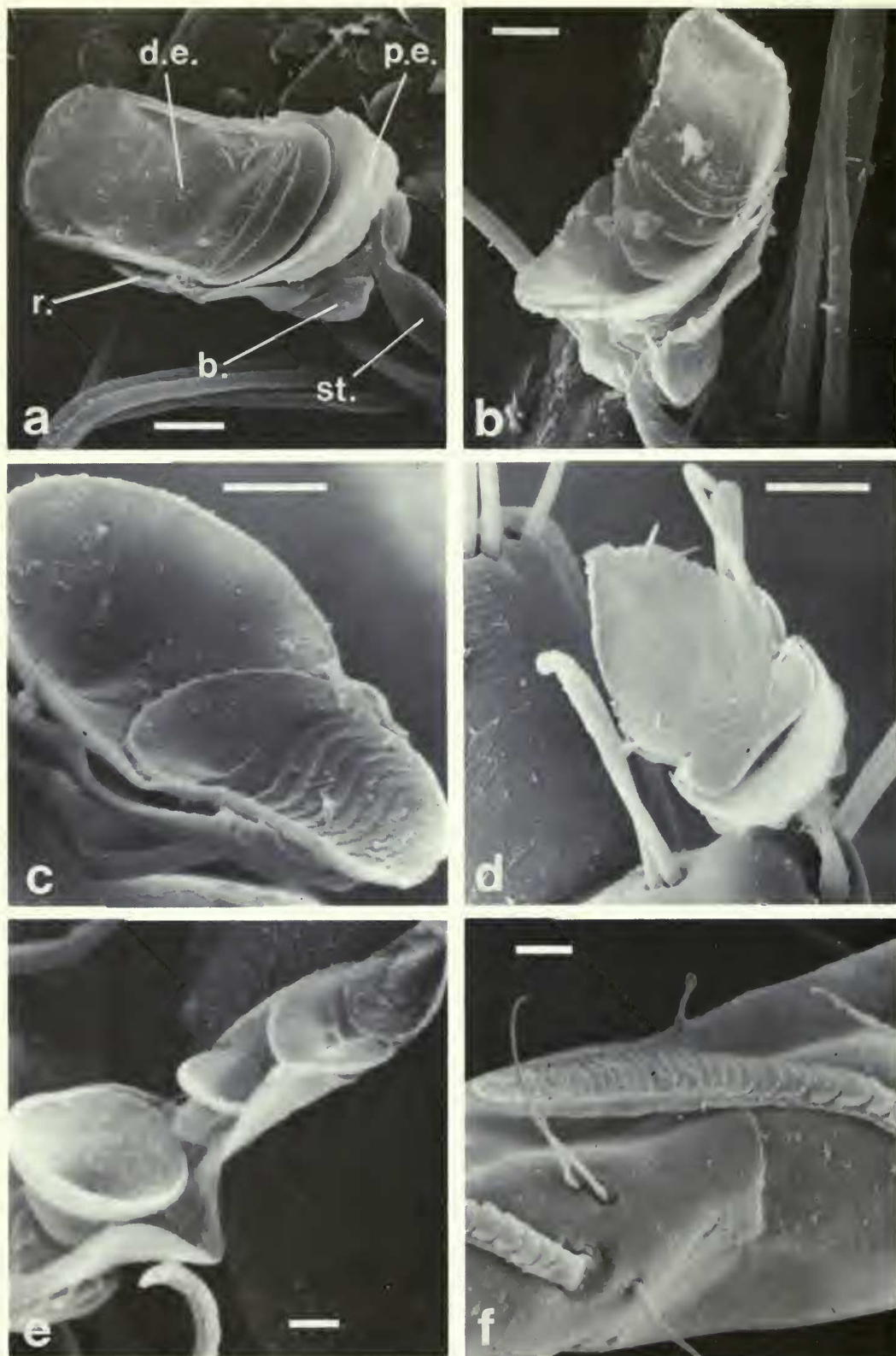


Fig. 3 a, *Calliopius laeviusculus*, d.e., distal element; p.e., proximal element; r., receptacle; b., bulla; st., stalk; b, *Paramoera gregaria*; c, *Eusiroides stenopleura*; d, *Apherusa jurinei*; e, *Chosroes incisus*; f, *Crangonyx pseudogracilis*. Bar scales a–d, f = 10 μ m, e = 2 μ m.

distal element. The banding of the pontogeneiid calceolus is usually transverse, sometimes weakly curved around a distal centre. Attachment of the surface elements to the elongate receptacle is like that in the lysianassid calceolus, and the bulla is similarly well developed.

5. Eusirid (Fig. 4a–d)

Eusirus antarcticus Thomson

Eusirus microps Walker

Eusirus perdentatus Chevreux

Rhachotropis aculeatus (Lepechin)

Rhachotropis helleri (Boeck)

Rhachotropis macropus Sars

Schraderia gracilis Pfeffer

Amathillopsis australis Stebbing

The special feature shared by the eusirid, gammarellid and oedicerotid types that immediately distinguishes them from other calceoli is the distinct separation of the proximal and distal elements and the remarkable cup-shaped configuration of the former. The proximal cup is robust, deeply concave, often set well apart from the distal element, and is attached to the receptacle only by a small basal connection. The following approximate measurements were obtained for the diameter of the proximal cup: *Eusirus antarcticus* 20–25 μm , *E. microps* 23–25 μm , *E. perdentatus* 25–60 μm , *Rhachotropis aculeatus* 45–70 μm , *R. helleri* 23–27 μm , *R. macropus* 23–30 μm , *Schraderia gracilis* 70 μm , *Amathillopsis australis* 25–40 μm . The distal element is elongated and carries a series of discrete crescentic plates, ranging from as few as 4 in *Rhachotropis* species and *Amathillopsis australis*, to 15 in *Eusirus antarcticus*, 25 in *Eusirus microps*, and more than 100 in *Eusirus perdentatus*. The multiplate distal element of the *Eusirus* species gives rise to an extremely elongate calceolus. The bulla at the base of the receptacle is pronounced in all eusirid calceoli.

Of all species studied the greatest development of the 'parabolic' proximal dish belongs to *Amathillopsis australis*. The largest calceolus was that sported by *Eusirus perdentatus*. The 'pore' in the apex of the distal element reported by Dahl (1975) for *Rhachotropis macropus* is not a true feature, but is an artefact produced by the rolling-up of the distal plate, probably the result of prolonged exposure to the electron beam or an excessive current.

We have included *Schraderia gracilis* in this group since it has a calceolus with an essentially eusirid-type design, although the structure of the surface elements is unusual. The proximal cup in particular is enormously enlarged and saucer-shaped extending well outside the supporting receptacle, and unlike those in other eusirids appears flexible with a frayed edge to its outer margin (The somewhat collapsed state of the proximal element may be an artefact of the s.e.m. preparation).

6. Gammarellid (Fig. 3e)

Gammarellus angulosus (Rathke)

Gammarellus homari (Fabricius)

Chosroes incisus Stebbing

The calceoli of these three species differ from the eusirid-type in the presence of a second cup-shaped element between the basic proximal and distal elements. Apart from this additional cup, the resemblance to the calceolus of *Rhachotropis* is quite strong. The proximal cup has a diameter of only about 8 μm in *Chosroes incisus* and 7 μm in *Gammarellus angulosus*, the intermediate cup measuring about 4.5 μm and 3.0 μm respectively.

Gammarellus and *Chosroes* are further united by the particular arrangement of the calceoli on the articles of the antennae. In both genera, the calceoli are situated in rows that extend all around the distal margins of the articles, unlike all other species examined in which calceoli are restricted to just one surface of the antenna. With the exception of *Urothoe*, the gammarellid calceoli were the smallest calceoli examined during this study.

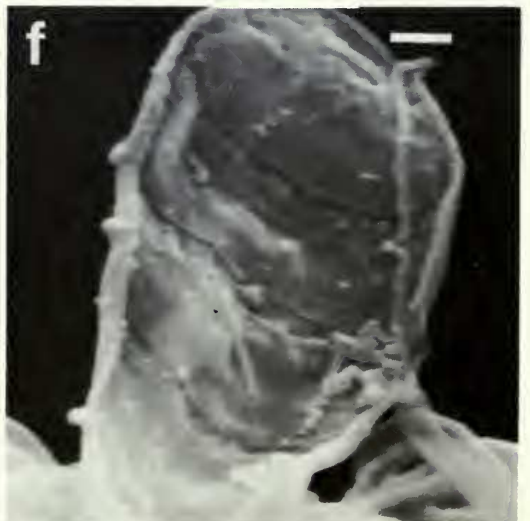
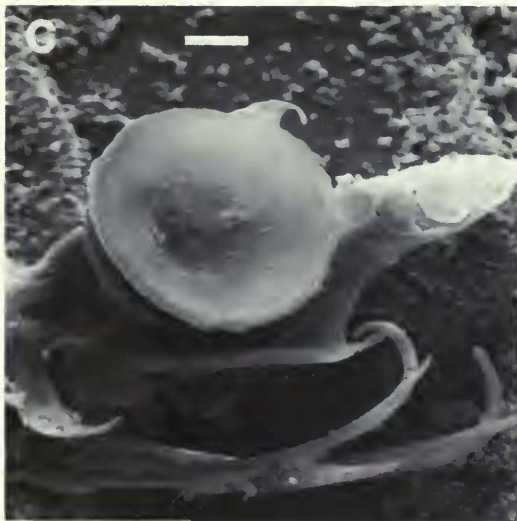
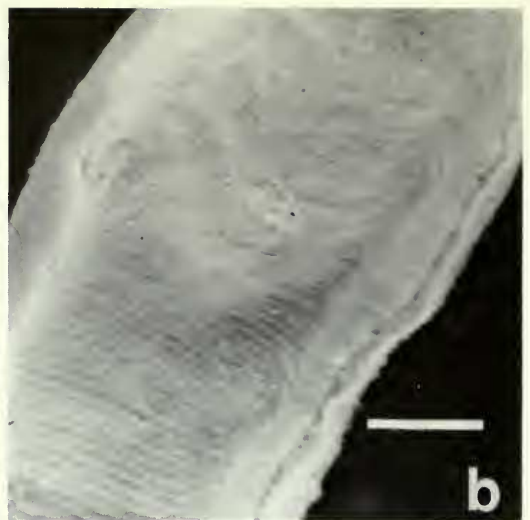


Fig. 4 a, *Eusirus antarcticus*; b, *Eusirus perdentatus*, mid part of distal element; c, *Amathillopsis australis*; d, *Amathillopsis australis*, proximal element; e, *Phoxocephalus regium*; f, *Urothoe elegans*. Bar scales a–e = 10 μ m, f = 2 μ m.

7. Oedicerotid (Fig. 1e)

Oediceroides calmani Walker*Oediceroides lahillei* Chevreux*Oediceropsis brevicornis* Liljeborg

A discrete proximal cup embraced by a broad lamellar receptacle, a small suboval distal element, and a waisted receptacle characterise the oedicerotid calceolus. The distal element, which has the distal half marked with distinct transverse ridges, is attached to the spatulate extension of the receptacle at the point of the slight surface depression. The bulla at the base of the receptacle is well developed. The proximal cup has a diameter of about 25–30 μm in *Oediceroides lahillei*.

8. Phoxocephalid (Fig. 4e, f)

Metaphoxus fultoni (Scott)*Metaphoxus pectinatus* Walker*Paraphoxus rostratus* (Dana)*Phoxocephalus regium* Barnard*Urothoe elegans* Bate

The phoxocephalid and crangonyctid calceoli differ in a number of ways from those already described although either could be derived from preceding types by a reduction in complexity.

In the phoxocephalid, the slender stalk and bulbous receptacle are absent and the surface elements are supported on a simple paddle-shaped lobe. No differentiated proximal element is apparent; instead the receptacle carries 3 to 6 oval, weakly concave plates which are probably homologous with the distal element of other calceoli. *Pontharpinia rostrata* has only 3 such plates of which the basal plate is much the largest and may represent the missing proximal element. There are 4 plates in *Phoxocephalus regium*, and 6 in *Metaphoxus pectinatus*, *M. fultoni*, and *Urothoe elegans*.

9. Crangonyctid (Fig. 3f)

Crangonyx pseudogracilis Bousfield*Synurella* sp.

The crangonyctid calceolus is a greatly extended version of the phoxocephalid design. Once again there is no discrete stalk or bulbous receptacle, but a paddle-shaped lobe supporting a series of narrow plates. The plates are crescent-shaped and separated one from another proximally, but become more closely packed and much narrower distally. There are about 20 plates in *Synurella* sp. and 35 in *Crangonyx pseudogracilis*.

We interpret the series of plates as the equivalent of the distal element of other calceoli, although the generally simplistic design of both the crangonyctid and phoxocephalid-types could indicate separate evolutionary development or developments.

Discussion

(a) Calceoli function

Although various suggestions have been made as to the function of calceoli the only direct experimental work of any importance is that of Dahl and colleagues in a series of controlled aquarium experiments devised to investigate the occurrence of pheromones in amphipods (Dahl *et al.*, 1970; Dahl, 1970, 1975). Adult females of *Gammarus duebeni* Liljeborg were fed a radioactive diet of ^3H labelled fish liver, and were introduced into an aquarium containing unlabelled male amphipods. The two sexes were kept apart by a fine nylon-mesh partition. After 30 and 60 minutes the amphipods were isolated and specimens selected for scintillation counting and microscope autoradiography. The males had by then become radioactive, and the ^3H label was localized on the second antenna, either within or very close to the calceoli. Dahl and colleagues concluded that a labelled pheromone produced by the female was dispersed in the aquarium water and taken up selectively either by the male

calceoli or by the tissue in the immediate vicinity of the calceoli. (The limited resolution of the microscope autoradiographic technique used did not permit precise localization of the uptake site). In *Gammarus duebeni* only males have calceoli.

It is our belief that the labelled pheromone may have been taken up by accompanying setae seen in Dahl's figures alongside the calceoli, perhaps indicated by the presence of two uptake sites in the same transverse section (Dahl *et al.*, 1970 Fig. 3A). The solitary nature of the calceoli in *duebeni* (Fig. 1a, b) would seem to preclude the presence of two calceoli in a single transverse or obliquely transverse section. Alternatively, the calceoli may secondarily provide an avenue for pheromone uptake that is incidental to their main function. We believe that they are structurally too complex for chemoreception to be their primary role. We note that chemoreceptors in Crustacea are typically simple sac-like structures (e.g. aesthetascs), or hair-like, or funnel-canals or pores (Barber, 1961), pegs or pits. The model of a protein sieve envisaged as the basis of an aquatic chemoreceptor does not demand the range of architectural novelty characteristic of calceoli. Calceoli have a morphological complexity greater than any other aquatic receptor of equivalent size that we have encountered in the literature.

The occurrence of calceoli on males and not on females is a common feature which suggests that calceoli have some involvement in the amphipod's reproductive behaviour. Calceoli are not always confined to one sex (Hurley, 1980) but when they are it is always the male that is calceoliferous. This is consistent with Dahl's pheromone theory, but since the vast majority of amphipod species are non-calceoliferous one would have to postulate that in these pheromone receptivity had been taken over by some other receptor, or that pheromones were not part of the behavioural strategy. We had hoped that a survey of the ecological and behavioural features of calceoliferous versus non-calceoliferous species would provide a clue to function but this was not the case. Calceoliferous species are found throughout almost the full spectrum of habitats characteristic of their family groups, and from what little is known about behaviour, calceoli can be present and absent in closely allied species apparently having similar habits and modes of life.

Other arguments against chemosensitivity as a primary role—admittedly based on comparative external arrangement only—are the orientation and directionality of calceoli. Calceoli are always arranged in one or more well defined rows along the axis of the antenna, normally the underside of antenna 1 and the upper surface of antenna 2. This arrangement permits a forwardly-directed 'array' of calceoli in an animal with antenna 1 raised and antenna 2 in a lowered posture. In addition, they are clearly organised to point in the same direction relative to the antennal axis. In some species, for example *Eusirus perdentatus* and *Amathillopsis australis*, although there is only one calceolus per segment they are ranged in repetitive pairs or triplets each slightly offset from its neighbour.

Directionality is a property of the calceolus itself and is most obvious in the 'parabolic' cup reminiscent of a radar reflector found in the most specialised forms. Searching for an explanation that is compatible with complexity, orientation and directionality we are drawn to one satisfying possibility—a sensitivity to water borne pressure waves whether produced by sound waves, animal vibrations or other disturbances in the water.

One could envisage the advantages of disturbance sensors in identifying the presence or approach of other animals, whether of the same species or not, in identifying movement-disturbances or behavioural characteristics of prey, or of water disturbances in streams around stones and ripples which would enable them to seek or avoid particular ecological situations. This sonar or phono-receptor theory is not supported by experimental evidence but we are hopeful that the photographs and discussion in this paper will attract the attention of biologists and in particular electrophysiologists with the experimental facilities to probe this possibility.

(b) Phylogenetic considerations

Despite the embryonic state of knowledge about phylogenetic relationships of higher gammaridean taxa it is noteworthy that the calceoliferous families have generally been

recognised as having some evolutionary affinity (Barnard, 1969; Bousfield, 1978). Notwithstanding the passing doubts occasioned by the structure of calceoli in Phoxocephaloidea and Crangonyctoidea it seems probable that calceoli have arisen only once in the Gammaridea and have undergone limited structural radiation during the evolution of the group. In the absence of evidence pointing to convergence, similarity of calceolus design may be taken as an indicator of geneological affinity.

The discovery of close structural similarities between the calceoli of many species traditionally placed in the same genus or family and discontinuities between species from different groups, has given us confidence that calceolus architecture has phylogenetic significance. Most of the species examined and allocated to the 9 calceolus-types are in good agreement with established family groupings but there are anomalies that suggest incorrect classification. Some of the species or genera which we considered wrongly designated during the early part of our study have since been relocated in a manner consistent with the calceoli evidence (Bousfield, 1978). The important anomalies are discussed below.

Amongst the first amphipods studied were species of *Bathyporeia* and *Urothoe*, two genera that for a long time have been placed together in the Haustoriidae. The calceoli are quite different, however, pointing to separate relationships, and it was further discovered that *Urothoe* shares the calceolus type of *Phoxocephalus*. This supports fully the recent revision by Bousfield (1978) in which *Urothoe* is moved from the haustoriids to a new family alongside *Phoxocephalus* in the superfamily Phoxocephaloidea. The bathyporeid-type calceolus is shared by *Zaramilla*, a genus placed in the Eusiridae by Barnard (1969), although special reference was made to its apparent 'haustoriid' affinities. There can be little doubt that *Zaramilla* belongs close to *Bathyporeia*, and the marked similarity of their calceoli to the gammarid-type (especially *Anisogammarus confervicolous*) must be further evidence for the proximity of the Pontoporeiidae to the gammaroid families.

Calceoli may prove particularly useful in re-assessing the Eusiridae, a family recently made very large and unwieldy by the inclusion of the families Pontogeneiidae and Calliopiidae (Barnard, 1969, 1972). Eusirid amphipods are frequently calceoliferous and have some of the largest and structurally most complex calceoli known. We have recognised 3 types within the eusirid complex—pontogeneiid, eusirid and gammarellid—and our allocation of species to each of these tends to cut across previously accepted family boundaries. Thus, the 'calliopiids' *Calliopioides*, *Apherusa*, *Halirages* and *Paracalliopoe* share the same type of calceolus as the 'pontogeneiid' *Pontogeneia*, and the 'eusirids' *Eusiroides*, *Eusiroopsis*, *Bovallia* and *Paramoera*. *Eusirus* and *Rhachotropis*, traditionally confamilial, must be joined by *Amathillopsis*, a genus having a chequered history being variously allocated to the Gammaridae, Amathillopsidae and the Paramphithoidae. We have placed *Schraderia* with our eusirids since it shares the same basic calceolus design, although it differs somewhat in detail and relative proportions.

The third group mentioned in the eusirid context, the gammarellid-type, brings together *Gammarellus* and *Chosroes*, linked by the common possession of an intermediate cup-shaped surface element. *Gammarellus* was, until its transfer to a new family (Bousfield, 1977) assigned to the Gammaridae, and *Chosroes* was with the calliopiids. If Bousfield's new family Gammarellidae receives general acceptance by amphipodologists, then *Chosroes* must be considered for inclusion also. It is particularly satisfying to note that, as well as having similar calceoli, *Gammarellus* and *Chosroes* (figured Sars, 1894; Stebbing, 1888) show a surprising similarity in many characters.

New perspectives produced by this SEM study should encourage other taxonomists to pay greater attention to this microscopic antennal receptor so often ignored in systematic descriptions, and, we hope, encourage some physiological work on their structure and function.

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